



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> OSTEOGENIC PEPTIDES  <b>(57) Abstract</b>  Disclosed are 1) the cDNA and amino acid sequences for novel polypeptide chains useful as subunits of dimeric osteogen- ic proteins, 2) osteogenic devices comprising these proteins in association with an appropriate carrier matrix, 3) methods of prod- ucing the polypeptide chains using recombinant DNA technology, and 4) methods of using the osteogenic devices to mimic the natural course of endochondral bone formation in mammals.		

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## Osteogenic peptides

### Background of the Invention

5        This invention relates to novel polypeptide chains  
and to osteogenic proteins comprising these polypeptide  
chains which are capable of inducing osteogenesis in  
mammals; to genes encoding the polypeptide chains; to  
10        methods for their production using recombinant DNA  
techniques, and to bone and cartilage repair procedures  
using the osteogenic proteins.

      Mammalian bone tissue is known to contain one or  
more proteinaceous materials, presumably active during  
15        growth and natural bone healing, which can induce a  
developmental cascade of cellular events resulting in  
endochondral bone formation. This active factor (or  
factors) has variously been referred to in the  
literature as bone morphogenetic or morphogenic  
20        protein, bone inductive protein, osteogenic protein,  
osteogenin, or osteoinductive protein.

      The developmental cascade of bone differentiation  
consists of recruitment of mesenchymal cells,  
25        proliferation of progenitor cells, calcification of  
cartilage, vascular invasion, bone formation,  
remodeling, and finally marrow differentiation (Reddi  
(1981) Collagen Rel. Res. 1:209-226).

30        Though the precise mechanisms underlying these  
phenotypic transformations are unclear, it has been  
shown that the natural endochondral bone  
differentiation activity of bone matrix can be  
dissociatively extracted and reconstituted with

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inactive residual collagenous matrix to restore full bone induction activity (Sampath and Reddi, (1981) Proc. Natl. Acad. Sci. USA 78:7599-7603). This provides an experimental method for assaying protein  
05 extracts for their ability to induce endochondral bone in vivo. Several species of mammals produce closely related protein as demonstrated by cross species implant experiments (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595).

10 The potential utility of these proteins has been recognized widely. It is contemplated that the availability of the protein would revolutionize orthopedic medicine, certain types of plastic surgery, and various periodontal and craniofacial reconstructive  
15 procedures.

The observed properties of these protein fractions have induced an intense research effort in various laboratories directed to isolating and identifying the pure factor or factors responsible for osteogenic  
20 activity. The current state of the art of purification of osteogenic protein from mammalian bone is disclosed by Sampath et al. ((1987) Proc. Natl. Acad. Sci. USA 84: 7109-7113). Urist et al. (1984) Proc. Soc. Exp. Biol. Med. 173: 194-199 disclose a human osteogenic  
25 protein fraction which was extracted from demineralized cortical bone by means of a calcium chloride-urea inorganic-organic solvent mixture, and retrieved by differential precipitation in guanidine-hydrochloride and preparative gel electrophoresis. The authors  
30 report that the protein fraction has an amino acid composition of an acidic polypeptide and a molecular weight in a range of 17-18 kD.

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Urist et al. (1984) Proc. Natl. Acad. Sci. USA 81:  
371-375 disclose a bovine bone morphogenetic protein  
extract having the properties of an acidic polypeptide  
and a molecular weight of approximately 18 kD. The  
05 authors reported that the protein was present in a  
fraction separated by hydroxyapatite chromatography,  
and that it induced bone formation in mouse hindquarter  
muscle and bone regeneration in trephine defects in rat  
and dog skulls. Their method of obtaining the extract  
10 from bone results in ill-defined and impure  
preparations.

European Patent Application Serial No. 148,155,  
published October 7, 1985, purports to disclose  
osteogenic proteins derived from bovine, porcine, and  
15 human origin. One of the proteins, designated by the  
inventors as a P3 protein having a molecular weight of  
22-24 kD, is said to have been purified to an  
essentially homogeneous state. This material is  
reported to induce bone formation when implanted into  
20 animals.

International Application No. PCT/087/01537,  
published January 14, 1988, discloses an impure  
fraction from bovine bone which has bone induction  
qualities. The named applicants also disclose putative  
25 "bone inductive factors" produced by recombinant DNA  
techniques. Four DNA sequences were retrieved from  
human or bovine genomic or cDNA libraries and expressed  
in recombinant host cells. While the applicants stated  
that the expressed proteins may be bone morphogenic  
30 proteins, bone induction was not demonstrated,  
suggesting that the recombinant proteins are not  
osteogenic. The same group reported subsequently  
(Science, 242:1528, Dec, 1988) that three of the four

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factors induce cartilage formation, and postulate that bone formation activity "is due to a mixture of regulatory molecules" and that "bone formation is most likely controlled ... by the interaction of these  
05 molecules." Again, no bone induction was attributed to the products of expression of the cDNAs. See also Urist et al., EP0,212,474 entitled Bone Morphogenic Agents.

Wang et al. (1988) Proc. Nat. Acad. Sci. USA 85:  
10 9484-9488 discloses the purification of a bovine bone morphogenetic protein from guanidine extracts of demineralized bone having cartilage and bone formation activity as a basic protein corresponding to a molecular weight of 30 kD determined from gel elution.  
15 Purification of the protein yielded proteins of 30, 18 and 16 kD which, upon separation, were inactive. In view of this result, the authors acknowledged that the exact identity of the active material had not been determined.

20 Wang et al. (1990) Proc. Nat. Acad. Sci. USA 87: 2220-2227 describes the expression and partial purification of one of the cDNA sequences described in PCT 87/01537. Consistent cartilage and/or bone formation with their protein requires a minimum of 600  
25 ng of 50% pure material.

International Application No. PCT/89/04458  
published April 19, 1990 (Int. Pub. No. WO90/003733), describes the purification and analysis of a family of osteogenic factors called "P3 OF 31-34". The protein  
30 family contains at least four proteins, which are characterized by peptide fragment sequences. The impure mixture P3 OF 31-34 is assayed for osteogenic

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05 activity. The activity of the individual proteins is  
neither assessed nor discussed.

It is an object of this invention to provide novel  
polypeptide chains useful as subunits of dimeric  
osteogenic proteins capable of endochondral bone  
10 formation in allogenic and xenogenic implants in  
mammals, including humans. Another object is to  
provide genes encoding these polypeptide chains and  
methods for the production of osteogenic proteins  
comprising these polypeptide chains using recombinant  
15 DNA techniques, as well as to provide antibodies  
capable of binding specifically to these proteins.

These and other objects and features of the  
invention will be apparent from the description,  
drawings, and claims which follow.

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Summary of the Invention

This invention provides novel polypeptide chains useful as either one or both subunits of dimeric osteogenic proteins which, when implanted in a  
05 mammalian body in association with a matrix, can induce at the locus of the implant the full developmental cascade of endochondral bone formation and bone marrow differentiation.

A key to these developments was the elucidation of  
10 amino acid sequence and structure data of native bovine osteogenic protein. A protocol was developed which results in retrieval of active, substantially pure osteogenic protein from bovine bone having a half-maximum bone forming activity of about 0.8 to 1.0 ng  
15 per mg of implant. The availability of the material enabled the inventors to elucidate key structural details of the protein necessary to achieve bone formation. Knowledge of the protein's amino acid sequence and other structural features enabled the  
20 identification and cloning of native genes in the human genome.

Consensus DNA sequences based on partial sequence data and observed homologies with regulatory proteins disclosed in the literature were used as probes for  
25 extracting genes encoding osteogenic protein from human genomic and cDNA libraries. One of the consensus sequences was used to isolate a previously unidentified gene which, when expressed, encoded a protein comprising a region capable of inducing endochondral  
30 bone formation when properly modified, incorporated in a suitable matrix, and implanted as disclosed herein. The gene, called "hOP1" or "OP-1" (human OP-1), is



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described in greater detail in copending U.S. 422,699, the disclosure of which is herein incorporated by reference.

In its native form, hOP1 expression yields an  
05 immature translation product ("hOP1-PP", where "PP"  
refers to "prepro form") of about 400 amino acids that  
subsequently is processed to yield a mature sequence of  
139 amino acids ("OP1-18"). The active region  
(functional domain) of the protein comprises the  
10 C-terminal 97 amino acids of the hOP1 sequence ("OPS").  
A long active sequence is OP7 (comprising the  
C-terminal 102 amino acids).

Further probing of mammalian cDNA libraries (human  
and mouse) with sequences specific to hOP1 also has  
15 identified novel OP1-like sequences herein referred to  
as "OP2" ("hOP2" or "mOP2"). The OP2 proteins share  
significant amino acid sequence homology, approximately  
74%, with the active region of the OP1 proteins (e.g.,  
OP7), and less homology with the intact mature form  
20 (e.g., OP1-18, 58% amino acid homology).

The amino acid sequence of the osteogenic proteins  
disclosed herein also share significant homology with  
various of the regulatory proteins on which the  
consensus probe was modeled. In particular, the  
25 proteins share significant homology in their C-terminal  
sequences, which comprise the active region of the  
osteogenic proteins. (Compare, for example, OP7 with  
DPP from *Drosophila* and Vgl from *Xenopus*. See, for  
example, U.S. Pat. No. 5,011,691). In addition, these  
30 proteins share a conserved six or seven cysteine  
skeleton in this region (e.g., the linear arrangement  
of these C-terminal cysteine residues is conserved in

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the different proteins.) See, for example, OP7, whose sequence defines the seven cysteine skeleton, or OPS, whose sequence defines the six cysteine skeleton. The OP2 proteins also contain an additional cysteine  
05 residue within this region.

Thus, in one preferred aspect, the invention comprises osteogenic proteins comprising a polypeptide chain comprising an amino acid sequence described by Seq. ID No. 3 or 5, including allelic and species  
10 variants thereof, and naturally-occurring or biosynthetic mutants, such that a dimeric protein comprising this polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a suitable  
15 matrix. Useful proteins include the full-length protein, mature proteins and truncated proteins comprising the functional domain described by the C-terminal.

In addition, the invention is not limited to these  
20 specific constructs. Thus, the osteogenic proteins of this invention comprising any of these polypeptide chains may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology  
25 which may be naturally occurring or biosynthetically derived, and active truncated or mutated forms of the native amino acid sequence, produced by expression of recombinant DNA in procaryotic or eucaryotic host cells. Active squances useful as osteogenic proteins  
30 of this invention are envisioned to include proteins capable of inducing endochondral bone formation when implanted in a mammal in association wiht a matrix and having at lest a 70% sequence homology, preferably at

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least 80%, with the amino acid sequence of OPS. This includes longer forms of a given protein, as well as allelic variants and muteins, including addition and deletion mutants, such as those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration still allows the protein to form a dimeric species having a conformation capable of inducing bone formation in a mammal when implanted in the mammal in association with a matrix.

The novel polypeptide chains and the osteogenic proteins they comprise can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and then purified, cleaved, refolded, dimerized, and implanted in experimental animals. Currently preferred host cells include E.coli or mammalian cells, such as CHO, COS or BSC cells. The osteogenic protein of the invention may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active proteins capable of inducing bone formation in mammals including humans. In view of this disclosure, those skilled in the art, using standard immunology

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techniques also may create antibodies capable of binding specifically to the osteogenic proteins disclosed herein, including fragments thereof.

The osteogenic proteins are useful in clinical applications in conjunction with a suitable delivery or support system (matrix). The matrix is made up of particles of porous materials. The pores must be of a dimension to permit progenitor cell migration and subsequent differentiation and proliferation. The particle size should be within the range of 70 - 850 mm, preferably 150mm - 420mm. It may be fabricated by close packing particulate material into a shape spanning the bone defect, or by otherwise structuring as desired a material that is biocompatible (non-inflammatory) and, biodegradable in vivo to serve as a "temporary scaffold" and substratum for recruitment of migratory progenitor cells, and as a base for their subsequent anchoring and proliferation. Currently preferred carriers include particulate, demineralized, guanidine extracted, species-specific (allogenic) bone, and specially treated particulate, protein extracted, demineralized, xenogenic bone. Optionally, such xenogenic bone powder matrices also may be treated with proteases such as trypsin and/or fibril modifying agents to increase the intraparticle intrusion volume and surface area. Useful agents include solvents such as dichloromethane, trichloroacetic acid, acetonitrile and acids such as trifluoroacetic acid and hydrogen fluoride. Alternatively, the matrix may be treated with a hot aqueous medium having a temperature within the range of about 37°C to 75°C, including a heated acidic aqueous medium. Other potentially useful matrix materials comprise collagen, homopolymers and copolymers of glycolic acid and lactic acid,

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hydroxyapatite, tricalcium phosphate and other calcium phosphates.

05 The osteogenic proteins and implantable osteogenic devices enabled and disclosed herein will permit the physician to obtain optimal predictable bone formation to correct, for example, acquired and congenital craniofacial and other skeletal or dental anomalies (Glowacki et al. (1981) Lancet 1:959-963). The devices may be used to induce local endochondral bone formation  
10 in non-union fractures as demonstrated in animal tests, and in other clinical applications including dental and periodontal applications where bone formation is required. Another potential clinical application is in cartilage repair, for example, in the treatment of  
15 osteoarthritis.

#### Brief Description of the Drawing

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following  
20 description, when read together with the accompanying drawings, in which:

FIGURE 1 compares the amino acid sequences of the mature mOP-2 and hOP-2 polypeptide chains: hOP2-A and mOP2-A; and

25 FIGURE 2 compares the amino acid sequences of the mature OP1 and OP2 polypeptide chains: OP1-18, mOP1-S, hOP2-A and mOP2-A.

#### Description

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Purification protocols first were developed which enabled isolation of the osteogenic protein present in crude protein extracts from mammalian bone. (See PCT WO 89/09787, published 19-OCT-89, and U.S. Serial No. 179,406 filed April 8, 1988, now U.S. Patent No. 4,968,950). The development of the procedure, coupled with the availability of fresh calf bone, enabled isolation of substantially pure bovine osteogenic protein (bOP). bOP was characterized significantly; its ability to induce cartilage and ultimately endochondral bone growth in cat, rabbit, and rat were demonstrated and studied; it was shown to be able to induce the full developmental cascade of bone formation previously ascribed to unknown protein or proteins in heterogeneous bone extracts. This dose dependent and highly specific activity was present whether or not the protein was glycosylated (see Sampath et al., (1990) J. Biol. Chem. 265: 13198-13205). Sequence data obtained from the bovine materials suggested probe designs which were used to isolate human genes. The OP human counterpart proteins have now been expressed and extensively characterized.

These discoveries enabled preparation of DNAs encoding totally novel, non-native protein constructs which individually as homodimers and combined with other species as heterodimers are capable of producing true endochondral bone (see PCT WO 09788, published 19-OCT-89, and US Serial No. 315,342, filed 23-FEB-89, now U.S. Patent No. 5,011,691). They also permitted expression of the natural material, truncated forms, muteins, analogs, fusion proteins, and various other variants and constructs, from cDNAs and genomic DNAs retrieved from natural sources or from synthetic DNA produced using the techniques disclosed herein and

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using automated, commercially available equipment. The DNAs may be expressed using well established molecular biology and recombinant DNA techniques in procaryotic or eucaryotic host cells, and may be oxidized and  
05 refolded in vitro if necessary, to produce biologically active protein.

One of the DNA sequences isolated from human genomic and cDNA libraries encoded a previously unidentified gene, referred to herein as OP1. The  
10 protein encoded by the isolated DNA was identified originally by amino acid homology with proteins in the TGF- $\beta$  family. Consensus splice signals were found where amino acid homologies ended, designating exon-intron boundaries. Three exons were combined to obtain  
15 a functional TGF- $\beta$ -like domain containing seven cysteines. (See, for example, U.S. Patent No. 5,011,691, or Ozkaynak, E. et al., (1990) EMBO. 9: 2085-2093).

The full-length cDNA sequence for hOP1, and its  
20 encoded "prepro" form "hOP1-PP," which includes an N-terminal signal peptide sequence, are disclosed in Seq. ID No. 1 (residues 1-431). The mature form of the hOP1 protein expressed in mammalian cells, "OP1-18", is described by amino acid residues 293-431 of Seq. ID  
25 No. 1. The full length form of hOP1, as well as various truncated forms of the gene, and fused genes, have been expressed in E. coli and numerous mammalian cells (see, for example, published PCT application WO 91/05802, published 2-MAY-91) and all have been shown  
30 to have osteogenic activity when implanted in a mammal in association with a suitable matrix.

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Given the foregoing amino acid and DNA sequence information, various nucleic acids (RNAs and DNAs) can be constructed which encode at least the active region of the hOP1 protein (e.g., OPS or OP7) and various  
05 analogs thereof (including allelic and species variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms of the mature proteins, and similar constructs. Moreover, DNA hybridization probes can be constructed from fragments  
10 of the hOP1 DNA or designed de novo based on the hOP1 DNA or amino acid sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional osteogenic proteins.

The DNAs can be produced by those skilled in the  
15 art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA  
20 Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks  
25 which may also be purified by PAGE.

DNAs used as hybridization probes may be labelled (e.g., as with a radioisotope, by nick-translation) and used to identify clones in a given library containing DNA to which the probe hybridizes, following techniques  
30 well known in the art. The libraries may be obtained commercially or they may constructed de novo using conventional molecular biology techniques. Further information on DNA library construction and



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hybridization techniques can be found in numerous texts known to those skilled in the art. See, for example, F.M. Ausubel., ed., Current Protocols in Molecular Biology-Vol. 1, (1989). In particular, see unit 5, 05 "Construction of Recombinant DNA Libraries" and Unit 6, "Screening of Recombinant Libraries."

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing 10 sequences of interest then can be transfected into an appropriate host cell for protein expression and further characterization. The host may be a procaryotic or eucaryotic cell since the former's inability to glycosylate protein will not destroy the 15 protein's osteogenic activity. Useful host cells include E. coli, Saccharomyces, the insect/baculovirus cell system, myeloma cells, and various mammalian cells. The vector additionally may encode various sequences to promote correct expression of the 20 recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like. The DNA sequence 25 encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant osteogenic protein also may be expressed as a fusion protein. After being translated, the protein 30 may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by oxidizing and refolding one or more of the various

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recombinant polypeptide chains within an appropriate eucaryotic cell or in vitro after expression of individual subunits. A detailed description of osteogenic protein expressed from recombinant DNA in E. coli is disclosed in U.S. Serial No. 660,162, filed 27-FEB-91, the disclosure of which incorporated by reference herein. A detailed description of osteogenic protein expressed from recombinant DNA in numerous different mammalian cells is disclosed in PCT WO91/05802, also incorporated herein by reference.

Finally, in view of the disclosure made herein, and using standard methodologies known in the art, persons skilled in the art can raise polyclonal and monoclonal antibodies against all or part of a polypeptide chain disclosed herein, such that the antibodies are capable of binding specifically to an epitope on the polypeptide chain. Useful protocols can be found in, for example, Molecular Cloning-A Laboratory Manual (Sambrook et al. eds., Cold Spring Harbor Press 2nd ed. 1989). See Book 3, Section 18.

#### Exemplification

In an effort to identify additional DNA sequences encoding osteogenic proteins, a hybridization probe specific to the C-terminus of the DNA of mature OP-1 was prepared using a StuI-EcoRI digest fragment of OP-1 (base pairs 1034-1354 in Sequence ID No. 1), and labelled with  $^{32}\text{P}$  by nick translation, as described in the art. As disclosed supra, the OP1 C-terminus encodes a key functional domain, e.g., the "active region" for osteogenic activity. The C-terminus also is the region of the protein whose amino acid sequence shares specific amino acid sequence homology with

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particular proteins in the TGF- $\beta$  super-family of regulatory proteins, and which includes the conserved cysteine skeleton.

Approximately  $7 \times 10^5$  phages of an oligo(dT) primed  
05 17.5 days p.c. mouse embryo 5' stretch cDNA (gt10)  
library (Clontech, Inc., Palo Alto, CA) was screened  
with the labelled probe. The screen was performed  
using the following stringent hybridization conditions:  
40% formamide, 5 x SSPE, 5 x Denhart's solution, 0.1%  
10 SDS, at 37°C overnight, and washing in 0.1 x SSPE, 0.1%  
SDS at 50°C.

Five recombinant phages were purified over three  
rounds of screening. Phage DNA was prepared from all  
five phages, subjected to an EcoRI digest, subcloned  
15 into the EcoRI site of a common pUC-type plasmid  
modified to allow single strand sequencing, and  
sequenced using means well known in the art.

Two different DNAs were identified by this  
procedure. One DNA, referred to herein as mOP1, has  
20 substantial homology to the mature form of OP1 (about  
98%), and is described in detail in copending USSN  
600,024, filed 18-Oct-90. A second DNA, encoding the  
C-terminus of a related gene and referred to herein as  
mOP2, also was identified by this procedure. The  
25 N-terminus of the gene encoding mOP2 was identified  
subsequently by screening a second mouse cDNA library  
(Mouse PCC4 cDNA (ZAP) library, Stratagene, Inc., La  
Jolla, CA).

Mouse OP2 (mOP2) protein shares significant amino  
30 acid sequence homology with the amino acid sequence of  
the hOP1 active region, e.g., OPS or OP7, about 74%

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homology, and less homology with the intact mature form, e.g., OP1-18, about 58% homology. The cDNA sequence, and the encoded amino acid sequence, for the full length mOP-2 protein is depicted in Sequence ID

05 No. 3. The full-length form of the protein is referred to as the prepro form of mOP-2 ("mOP2-PP"), and includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 3) is believed

10 to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Ala-Pro-Arg-Ala (amino acid residues 255-259 of Sequence ID

15 No. 3) is believed to constitute the cleavage site that produces the mature form of the protein, herein referred to as "mOP2-A", and described by residues 259-397 of Seq. ID No. 3. Residues 301-397 of Seq. ID No. 3 correspond to the region defining the conserved

20 six cysteine skeleton. Residues 296-397 of Seq. ID No. 3 correspond to the region defining the conserved seven cysteine skeleton.

Using a probe prepared from the pro region of mOP2 (an EcoRI-BamHI digest fragment, bp 467-771 of Sequence

25 ID No. 3), a human hippocampus library was screened (human hippocampus cDNA lambda (ZAP II library Stratagene, Inc., La Jolla, CA) following essentially the same procedure as for the mouse library screens. The procedure identified the N-terminus of a novel DNA

30 encoding an amino acid sequence having substantial homology with mOP2. The C-terminus of the gene subsequently was identified by probing a human genomic library (in lambda phage EMBL-3, Clontech, Inc., Palo Alto, CA) with a labelled fragment from the novel human

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05 DNA in hand. The novel polypeptide chain encoded by this DNA is referred to herein as hOP2 protein, and shares almost complete amino acid identity (about 92% amino acid sequence homology) with mOP2-A (see Fig. 1 and infra).

10 The cDNA sequence, and the encoded amino acid sequence, for the prepro form of hOP2, "hOP2-PP", is described in Sequence ID No. 5. This full-length form of the protein also includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 5) is believed to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Thr-Pro-Arg-Ala (amino acid residues 257-261 of Sequence ID No. 5) is believed to constitute the cleavage site that produces what is believed to be the mature form of the protein, herein referred to as hOP2-A" and described by residues 261-399 of Seq. ID No. 5.

25 Additional mature species of hOP2 thought to be active include truncated sequences, "hOP2-P" (described by residues 264-399 of Seq. ID No. 5) and "hOP2-R" (described by residues 267-399 of Seq. ID No. 5), and a slightly longer sequence ("hOP2-S", described by residues 240-399 of Seq. ID No. 5). Residues 303-399 of Seq. ID No. 5 correspond to the region defining the conserved six cysteine skeleton. Residues 297-399 of Seq. ID No. 5 correspond to the region defining the conserved seven cystein skeleton.

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It should be noted that the nucleic acid sequence encoding the N-terminus of the prepro form of both mOP2 and hOP2 is rich in guanidine and cytosine base pairs. As will be appreciated by those skilled in the art, sequencing such a "G-C rich" region can be problematic, due to stutter and/or band compression. Accordingly, the possibility of sequencing errors in this region can not be ruled out. However, the definitive amino acid sequence for these and other, similarly identified proteins can be determined readily by expressing the protein from recombinant DNA using, for example, any of the means disclosed herein, and sequencing the polypeptide chain by conventional peptide sequencing methods well known in the art.

Figure 1 compares the amino acid sequences of mature mOP2 and hOP2. Identity is indicated by three dots (...) in the mOP2 sequence. As is evident from the figure, the amino acid sequence homology between the mature forms of these two proteins is substantial (92% homology between the mature sequences, about 95% homology within the C-terminal active region (e.g., residues 38-139 or 42-139 of Fig. 1.)

Fig. 2 compares the amino acid sequences for the mature forms of all four species of OP1 and OP2 proteins. Here again, identity is indicated by three dots (...). Like the mOP2 protein, the hOP2 protein shares significant homology (about 74%) with the amino acid sequence defining the OP1 active region (OPS or OP7, residues 43-139 and 38-139, respectively, in Fig. 2), and less homology with OP1-18 (about 58% homology). Both OP2 proteins share the conserved seven cysteine skeleton seen in the OP1 proteins. In

- 21 -

addition, the OP2 proteins comprise an eighth cysteine residue within this region (see position 78 in FIG. 2).

A preferred generic amino acid sequence useful as a subunit of a dimeric osteogenic protein capable of inducing endochondral bone or cartilage formation when implanted in a mammal in association with a matrix, and which incorporates the maximum homology between the identified OP1 and OP2 proteins, can be described by the sequence referred to herein as "OPX", described below and in Seq. No.7.

	Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe	
	1	5 10
15	Xaa Asp Leu Gly Trp Xaa Asp Trp Xaa Ile	
		15 20
	Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys	
		25 30
20	Glu Gly Glu Cys Xaa Phe Pro Leu Xaa Ser	
		35 40
	Xaa Met Asn Ala Thr Asn His Ala Ile Xaa	
		45 50
	Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa	
		55 60
25	Xaa Val Pro Lys Xaa Cys Cys Ala Pro Thr	
		65 70
	Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa	
		75 80
30	Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys	
		85 90
	Xaa Arg Asn Met Val Val Xaa Ala Cys Gly	
		95 100
	Cys His,	

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and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 9 = (Ser or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 =  
5 (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at  
10 res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 =  
15 (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 87 = (Ile or Asp); Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr, Ala or His); and Xaa at res. 97 = (Arg or Lys).

20 The high degree of homology exhibited between the various OP1 and OP2 proteins suggests that the novel osteogenic proteins identified herein will purify essentially as OP1 does, or with only minor modifications of the protocols disclosed for OP1.

25 Similarly, the purified mOP1, mOP2, and hOP2 proteins are predicted to have an apparent molecular weight of about 18 kDa as reduced single subunits, and an apparent molecular weight of about 36 kDa as oxidized dimers, as determined by comparison with molecular

30 weight standards on an SDS-polyacrylamide electrophoresis gel. Unglycosylated dimers (e.g., proteins produced by recombinant expression in *E. coli*) are predicted to have an apparent molecular weight of about 27 kDa. There appears to be one potential N





- 24 -

```

          35              40
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
  45              50              55
Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa
05              60              65
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              70              75
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              80              85
10 Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys Xaa
          90              95

```

"OPX-8C" (Sequence ID No. 9 comprising additional five residues at the N-terminus, including a conserved cysteine residue):

```

15 Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
   1              5              10
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          15              20
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
20          25              30
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa
          35              40              45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          50              55
25 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
          60              65
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          70              75
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          80              85
30 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
          90              95
Xaa Cys Xaa

```

100

## MATRIX PREPARATION

## A. General Consideration of Matrix Properties

05 The currently preferred carrier material is a  
xenogenic bone-derived particulate matrix treated as  
disclosed herein. This carrier may be replaced by  
either a biodegradable-synthetic or synthetic-inorganic  
matrix (e.g., hydroxylapatite (HAP), collagen,  
tricalcium phosphate or polylactic acid, polyglycolic  
10 acid and various copolymers thereof.)

Studies have shown that surface charge, particle  
size, the presence of mineral, and the methodology for  
combining matrix and osteogenic protein all play a role  
in achieving successful bone induction. Perturbation  
15 of the charge by chemical modification abolishes the  
inductive response. Particle size influences the  
quantitative response of new bone; particles between  
75  $\mu\text{m}$  and 420  $\mu\text{m}$  elicit the maximum response.  
Contamination of the matrix with bone mineral will  
20 inhibit bone formation. Most importantly, the  
procedures used to formulate OP onto the matrix are  
extremely sensitive to the physical and chemical state  
of both the osteogenic protein and the matrix.

The sequential cellular reactions in the  
25 interface of the bone matrix/osteogenic protein  
implants are complex. The multistep cascade includes:  
binding of fibrin and fibronectin to implanted matrix,  
chemotaxis of cells, proliferation of fibroblasts,  
differentiation into chondroblasts, cartilage

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formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

A successful carrier for osteogenic protein must perform several important functions. It must bind  
05 osteogenic protein and act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the osteogenic protein from nonspecific proteolysis. In addition,  
10 selected materials must be biocompatible in vivo and preferably biodegradable; the carrier must act as a temporary scaffold until replaced completely by new bone. Polylactic acid (PLA), polyglycolic acid (PGA), and various combinations have different dissolution rates in vivo. In bones, the dissolution rates can  
15 vary according to whether the implant is placed in cortical or trabecular bone.

Matrix geometry, particle size, the presence of surface charge, and the degree of both intra-and-inter-particle porosity are all important to successful  
20 matrix performance. It is preferred to shape the matrix to the desired form of the new bone and to have dimensions which span non-union defects. Rat studies show that the new bone is formed essentially having the dimensions of the device implanted.

The matrix may comprise a shape-retaining  
25 solid made of loosely adhered particulate material, e.g., with collagen. It may also comprise a molded, porous solid, or simply an aggregation of close-packed particles held in place by surrounding tissue.  
30 Masticated muscle or other tissue may also be used. Large allogenic bone implants can act as a carrier for the matrix if their marrow cavities are cleaned and

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packed with particle and the dispersed osteogenic protein.

The preferred matrix material, prepared from xenogenic bone and treated as disclosed herein, produces an implantable material useful in a variety of clinical settings. In addition to its use as a matrix for bone formation in various orthopedic, periodontal, and reconstructive procedures, the matrix also may be used as a sustained release carrier, or as a collagenous coating for implants. The matrix may be shaped as desired in anticipation of surgery or shaped by the physician or technician during surgery. Thus, the material may be used for topical, subcutaneous, intraperitoneal, or intramuscular implants; it may be shaped to span a nonunion fracture or to fill a bone defect. In bone formation or conduction procedures, the material is slowly absorbed by the body and is replaced by bone in the shape of or very nearly the shape of the implant.

Various growth factors, hormones, enzymes, therapeutic compositions, antibiotics, and other body treating agents also may be absorbed onto the carrier material and will be released over time when implanted as the matrix material is slowly absorbed. Thus, various known growth factors such as EGF, PDGF, IGF, FGF, TGF- $\alpha$ , and TGF- $\beta$  may be released in vivo. The material can be used to release chemotherapeutic agents, insulin, enzymes, or enzyme inhibitors.

## B. Bone-Derived Matrices

### 1. Preparation of Demineralized Bone

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Demineralized bone matrix, preferably bovine bone matrix, is prepared by previously published procedures (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595). Bovine diaphyseal bones (age 05 1-10 days) are obtained from a local slaughterhouse and used fresh. The bones are stripped of muscle and fat, cleaned of periosteum, demarrowed by pressure with cold water, dipped in cold absolute ethanol, and stored at -20°C. They are then dried and fragmented by crushing 10 and pulverized in a large mill. Care is taken to prevent heating by using liquid nitrogen. The pulverized bone is milled to a particle size in the range of 70-850  $\mu\text{m}$ , preferably 150-420  $\mu\text{m}$ , and is defatted by two washes of approximately two hours 15 duration with three volumes of chloroform and methanol (3:1). The particulate bone is then washed with one volume of absolute ethanol and dried over one volume of anhydrous ether yielding defatted bone powder. The defatted bone powder is then demineralized by four 20 successive treatments with 10 volumes of 0.5 N HCl at 4°C for 40 min. Finally, neutralizing washes are done on the demineralized bone powder with a large volume of water.

## 2. Guanidine Extraction

25 Demineralized bone matrix thus prepared is extracted with 5 volumes of 4 M guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hr. at 4°C. The suspension is filtered. The insoluble material is collected and used to fabricate the matrix. The material is mostly 30 collagenous in nature. It is devoid of osteogenic or chondrogenic activity.

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### 3. Matrix Treatments

The major component of all bone matrices is Type-I collagen. In addition to collagen, demineralized bone extracted as disclosed above includes non-collagenous proteins which may account for 5% of its mass. In a xenogenic matrix, these noncollagenous components may present themselves as potent antigens, and may constitute immunogenic and/or inhibitory components. These components also may inhibit osteogenesis in allogenic implants by interfering with the developmental cascade of bone differentiation. It has been discovered that treatment of the matrix particles with a collagen fibril-modifying agent extracts potentially unwanted components from the matrix, and alters the surface structure of the matrix material. Useful agents include acids, organic solvents or heated aqueous media. Various treatments are described below. A detailed physical analysis of the effect these fibril-modifying agents have on demineralized, guanidine-extracted bone collagen particles is disclosed in PCT WO 90/10018, published 7-SEP-90.

After contact with the fibril-modifying agent, the treated matrix is washed to remove any extracted components, following a form of the procedure set forth below:

1. Suspend in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) or water and stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);

- 30 -

2. Centrifuge and repeat wash step; and

3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

### 3.1 Acid Treatments

05 1. Trifluoroacetic acid.

Trifluoroacetic acid is a strong non-oxidizing acid that is a known swelling agent for proteins, and which modifies collagen fibrils.

10 Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. These particles are extracted with various percentages (1.0% to 100%) of trifluoroacetic acid and water (v/v) at 0°C or room temperature for 1-2 hours with constant stirring. The treated matrix is  
15 filtered, lyophilized, or washed with water/salt and then lyophilized.

2. Hydrogen Fluoride.

Like trifluoroacetic acid, hydrogen fluoride is a strong acid and swelling agent, and also is  
20 capable of altering intraparticle surface structure. Hydrogen fluoride is also a known deglycosylating agent. As such, HF may function to increase the osteogenic activity of these matrices by removing the antigenic carbohydrate content of any glycoproteins  
25 still associated with the matrix after guanidine extraction.



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Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. The sample is dried in vacuo over  $P_2O_5$ , transferred to the reaction vessel and exposed to  
05 anhydrous hydrogen fluoride (10-20 ml/g of matrix) by distillation onto the sample at  $-70^\circ\text{C}$ . The vessel is allowed to warm to  $0^\circ\text{C}$  and the reaction mixture is stirred at this temperature for 120 minutes. After evaporation of the hydrogen fluoride in vacuo, the  
10 residue is dried thoroughly in vacuo over KOH pellets to remove any remaining traces of acid. Extent of deglycosylation can be determined from carbohydrate analysis of matrix samples taken before and after treatment with hydrogen fluoride, after washing the  
15 samples appropriately to remove non-covalently bound carbohydrates. SDS-extracted protein from HF-treated material is negative for carbohydrate as determined by Con A blotting.

The deglycosylated bone matrix is next washed  
20 twice in TBS (Tris-buffered saline) or UTBS, water-washed, and then lyophilized.

Other acid treatments are envisioned in addition to HF and TFA. TFA is a currently preferred acidifying reagent in these treatments because of its  
25 volatility. However, it is understood that other, potentially less caustic acids may be used, such as acetic or formic acid.

### 3.2 Solvent Treatment

#### 1. Dichloromethane.

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Dichloromethane (DCM) is an organic solvent capable of denaturing proteins without affecting their primary structure. This swelling agent is a common reagent in automated peptide synthesis, and is used in 05 washing steps to remove components.

Bovine bone residue, prepared as described above, is sieved, and particles of the appropriate size are incubated in 100% DCM or, preferably, 99.9% DCM/0.1% TFA. The matrix is incubated with the 10 swelling agent for one or two hours at 0°C or at room temperature. Alternatively, the matrix is treated with the agent at least three times with short washes (20 minutes each) with no incubation.

## 2. Acetonitrile.

15 Acetonitrile (ACN) is an organic solvent, capable of denaturing proteins without affecting their primary structure. It is a common reagent used in high-performance liquid chromatography, and is used to elute proteins from silica-based columns by perturbing 20 hydrophobic interactions.

Bovine bone residue particles of the appropriate size, prepared as described above, are treated with 100% ACN (1.0 g/30 ml) or, preferably, 99.9% ACN/0.1% TFA at room temperature for 1-2 hours 25 with constant stirring. The treated matrix is then water-washed, or washed with urea buffer, or 4 M NaCl and lyophilized. Alternatively, the ACN or ACN/TFA treated matrix may be lyophilized without wash.

## 3. Isopropanol.

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Isopropanol is also an organic solvent capable of denaturing proteins without affecting their primary structure. It is a common reagent used to elute proteins from silica HPLC columns.

05           Bovine bone residue particles of the appropriate size prepared as described above are treated with 100% isopropanol (1.0 g/30 ml) or, preferably, in the presence of 0.1% TFA, at room temperature for 1-2 hours with constant stirring. The  
10 matrix is then water-washed or washed with urea buffer or 4 M NaCl before being lyophilized.

#### 4. Chloroform

Chloroform also may be used to increase surface area of bone matrix like the reagents set forth  
15 above, either alone or acidified.

Treatment as set forth above is effective to assure that the material is free of pathogens prior to implantation.

#### 3.3 Heat Treatment

20           The currently most preferred agent is a heated aqueous fibril-modifying medium such as water, to increase the matrix particle surface area and porosity. The currently most preferred aqueous medium is an acidic aqueous medium having a pH of less than about  
25 4.5, e.g., within the range of pH 2 - pH 4. which may help to "swell" the collagen before heating. 0.1% acetic acid, which has a pH of about 3, currently is preferred. 0.1 M acetic acid also may be used.

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Various amounts of delipidated, demineralized guanidine-extracted bone collagen are heated in the aqueous medium (1g matrix/30ml aqueous medium) under constant stirring in a water jacketed glass flask, and  
05 maintained at a given temperature for a predetermined period of time. Preferred treatment times are about one hour, although exposure times of between about 0.5 to two hours appear acceptable. The temperature employed is held constant at a temperature generally  
10 within the range of about 37°C to 75°C. The currently preferred heat treatment temperature is within the range of 45°C to 60°C.

After the heat treatment, the matrix is filtered, washed, lyophilized and used for implant. Where an  
15 acidic aqueous medium is used, the matrix also is preferably neutralized prior to washing and lyophilization. A currently preferred neutralization buffer is a 200mM sodium phosphate buffer, pH 7.0. To neutralize the matrix, the matrix preferably first is  
20 allowed to cool following thermal treatment, the acidic aqueous medium (e.g., 0.1% acetic acid) then is removed and replaced with the neutralization buffer and the matrix agitated for about 30 minutes. The neutralization buffer then may be removed and the  
25 matrix washed and lyophilized (see infra).

The matrix also may be treated to remove contaminating heavy metals, such as by exposing the matrix to a metal ion chelator. For example, following thermal treatment with 0.1% acetic acid, the matrix may  
30 be neutralized in a neutralization buffer containing EDTA (sodium ethylenediaminetetraacetic acid), e.g., 200 mM sodium phosphate, 5mM EDTA, pH 7.0. 5 mM EDTA provides about a 100-fold molar excess of chelator to

- 35 -

residual heavy metals present in the most contaminated matrix tested to date. Subsequent washing of the matrix following neutralization appears to remove the bulk of the EDTA. EDTA treatment of matrix particles  
05 reduces the residual heavy metal content of all metals tested (Sb, As, Be, Cd, Cr, Cu, Co, Pb, Hg, Ni, Se, Ag, Zn, Tl) to less than about 1 ppm. Bioassays with EDTA-treated matrices indicate that treatment with the metal ion chelator does not inhibit bone inducing activity.

10 The collagen matrix materials preferably take the form of a fine powder, insoluble in water, comprising nonadherent particles. It may be used simply by packing into the volume where new bone growth or sustained release is desired, held in place by  
15 surrounding tissue. Alternatively, the powder may be encapsulated in, e.g., a gelatin or polylactic acid coating, which is adsorbed readily by the body. The powder may be shaped to a volume of given dimensions and held in that shape by interadhering the particles  
20 using, for example, soluble, species-biocompatible collagen. The material may also be produced in sheet, rod, bead, or other macroscopic shapes.

Demineralized rat bone matrix used as an allogenic matrix in certain of the experiments  
25 disclosed herein, is prepared from several of the dehydrated diaphyseal shafts of rat femur and tibia as described herein to produce a bone particle size which passes through a 420  $\mu$ m sieve. The bone particles are subjected to dissociative extraction with 4 M  
30 guanidine-HCl. Such treatment results in a complete loss of the inherent ability of the bone matrix to induce endochondral bone differentiation. The remaining insoluble material is used to fabricate the

- 36 -

matrix. The material is mostly collagenous in nature, and upon implantation, does not induce cartilage and bone. All new preparations are tested for mineral content and osteogenic activity before use. The total  
05 loss of biological activity of bone matrix is restored when an active osteoinductive protein fraction or a pure osteoinductive protein preparation is reconstituted with the biologically inactive insoluble collagenous matrix.

10

## FABRICATION OF OSTEOGENIC DEVICE

The naturally sourced and recombinant protein as set forth above, and other constructs, can be combined and dispersed in a suitable matrix preparation using any of the methods described below. In general, 50-100  
15 ng of active protein is combined with the inactive carrier matrix (e.g., 25 mg for rat bioassays). Greater amounts may be used for large implants.

## 1. Ethanol Precipitation

Matrix is added to osteogenic protein  
20 dissolved in guanidine-HCl. Samples are vortexed and incubated at a low temperature (e.g., 4°C). Samples are then further vortexed. Cold absolute ethanol (5 volumes) is added to the mixture which is then stirred and incubated, preferably for 30 minutes at -20°C.  
25 After centrifugation (microfuge, high speed) the supernatant is discarded. The reconstituted matrix is washed twice with cold concentrated ethanol in water (85% EtOH) and then lyophilized.

## 2. Acetonitrile Trifluoroacetic

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### Acid Lyophilization

In this procedure, osteogenic protein in an acetonitrile trifluoroacetic acid (ACN/TFA) solution is added to the carrier material. Samples are vigorously  
05 vortexed many times and then lyophilized. This method is currently preferred, and has been tested with osteogenic protein at varying concentrations and different levels of purity.

### 3. Urea Lyophilization

10 For those osteogenic proteins that are prepared in urea buffer, the protein is mixed with the matrix material, vortexed many times, and then lyophilized. The lyophilized material may be used "as is" for implants.

### 15 4. Buffered Saline Lyophilization

OP1 and OP2 preparations in physiological saline may also be vortexed with the matrix and lyophilized to produce osteogenically active material.

20 These procedures also can be used to adsorb other active therapeutic drugs, hormones, and various bioactive species to the matrix for sustained release purposes.

### BIOASSAY

The functioning of the various proteins and  
25 devices of this invention can be evaluated with an in vivo bioassay. Studies in rats show the osteogenic effect in an appropriate matrix to be dependent on the

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dose of osteogenic protein dispersed in the matrix. No activity is observed if the matrix is implanted alone. In vivo bioassays performed in the rat model also have shown that demineralized, guanidine-extracted xenogenic bone matrix materials of the type described in the literature are ineffective as a carrier, fail to induce bone, and produce an inflammatory and immunological response when implanted unless treated as disclosed above. In certain species (e.g., monkey) allogenic matrix materials also apparently are ineffective as carriers. The following sets forth various procedures for preparing osteogenic devices from the proteins and matrix materials prepared as set forth above, and for evaluating their osteogenic utility.

15 A. Rat Model

1. Implantation

The bioassay for bone induction as described by Sampath and Reddi ((1983) Proc. Natl. Acad. Sci. USA 80 6591-6595), herein incorporated by reference, may be used to monitor endochondral bone differentiation activity. This assay consists of implanting test samples in subcutaneous sites in recipient rats under ether anesthesia. Male Long-Evans rats, aged 28-32 days, were used. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. Approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The day of implantation is designated as day one of the experiment. Implants were removed on day 12. The heterotopic site allows for the study of bone induction without the possible



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ambiguities resulting from the use of orthotropic sites. As disclosed herein, both allogenic (rat bone matrix) and xenogenic (bovine bone matrix) implants were assayed.

## 05 2. Cellular Events

Successful implants exhibit a controlled progression through the stages of protein-induced endochondral bone development, including: (1) transient infiltration by polymorphonuclear leukocytes on day  
10 one; (2) mesenchymal cell migration and proliferation on days two and three; (3) chondrocyte appearance on days five and six; (4) cartilage matrix formation on day seven; (5) cartilage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and  
15 formation of new bone on days nine and ten; (7) appearance of osteoblastic and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and (8) hematopoietic bone marrow  
20 differentiation in the ossicle on day twenty-one. The results show that the shape of the new bone conforms to the shape of the implanted matrix.

## 3. Histological Evaluation

Histological sectioning and staining is preferred to determine the extent of osteogenesis in  
25 the implants. Implants are fixed in Bouins Solution, embedded in paraffin, and cut into 6-8  $\mu$ m sections. Staining with toluidine blue or hemotoxylin/eosin demonstrates clearly the ultimate development of endochondral bone. Twelve day implants are usually  
30 sufficient to determine whether the implants contain newly induced bone.

- 40 -

#### 4. Biological Markers

Alkaline phosphatase activity may be used as a marker for osteogenesis. The enzyme activity may be determined spectrophotometrically after homogenization  
05 of the implant. The activity peaks at 9-10 days in vivo and thereafter slowly declines. Implants showing no bone development by histology have little or no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and  
10 obtaining an estimate of bone formation quickly after the implants are removed from the rat. Alternatively, the amount of bone formation can be determined by measuring the calcium content of the implant.

The invention may be embodied in other specific  
15 forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather  
20 than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 05 (i) APPLICANT: OPPERMAN, HERMANN  
OZKAYNAK, ENGIN  
KUBERASAMPATH, THANGAVEL  
RUEGER, DAVID C.
- (ii) TITLE OF INVENTION: OSTEOGENIC DEVICES
- (iii) NUMBER OF SEQUENCES: 9
- 10 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: TESTA, HURWITZ & THIBEAULT  
(B) STREET: 53 STATE STREET  
(C) CITY: BOSTON  
(D) STATE: MASSACHUSETTS  
15 (E) COUNTRY: U.S.A.  
(F) ZIP: 02109
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 25 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: PITCHER, EDMUND R.  
(B) REGISTRATION NUMBER: 27,829  
(C) REFERENCE/DOCKET NUMBER: CRR056PC
- 30 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 617/248-7000  
(B) TELEFAX: 617/248-7100

## (2) INFORMATION FOR SEQ ID NO:1:

- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1822 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(F) TISSUE TYPE: HIPPOCAMPUS

05 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 49..1341

(C) IDENTIFICATION METHOD: experimental

10 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "hOP1-PP"  
 /evidence= EXPERIMENTAL  
 /standard\_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

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15                                     Met His Val
                                     1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA      105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
      5                      10                      15

20 CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC      153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
      20                      25                      30                      35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG      201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
25                      40                      45                      50

CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC      249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
                      55                      60                      65

30 CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG      297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
                      70                      75                      80

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC      345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
      85                      90                      95

35 GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC      393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
100                      105                      110                      115

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC      441
Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp

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					120					125					130		
					ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC												489
					Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe												
					135					140					145		
05	CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC																537
	His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile																
					150					155					160		
	CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC																585
10	Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp																
					165					170					175		
	TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT																633
	Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr																
					180					185					190		195
	CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC																681
15	Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu																
					200					205					210		
	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC																729
	Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp																
					215					220					225		
20	ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG																777
	Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu																
					230					235					240		
	GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC																825
25	Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro																
					245					250					255		
	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC																873
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro																
					260					265					270		275
	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC																921
30	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile																
					280					285					290		
	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC																969
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro																
					295					300					305		
35	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC																1017
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser																
					310					315					320		
	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC																1065
40	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe																
					325					330					335		

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05	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
	340 345 350 355	
	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
10	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
	360 365 370	
	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
	375 380 385	
15	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257
	Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala	
	390 395 400	
	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305
	Ile Ser Val Leu Tyr Phe Asp Ser Ser Asn Val Ile Leu Lys Lys	
	405 410 415	
20	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC	1351
	Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His	
	420 425 430	
	GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
	GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG	1471
25	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531
	ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC	1591
	GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT	1651
	CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG	1711
	GGCGTGGCAA GGGGTGGGCA CATTGGTGTG TGTGCGAAAG GAAAATTGAC CCGGAAGTTC	1771
30	CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A	1822

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 431 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

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05

(D) OTHER INFORMATION: /Product="hOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met	His	Val	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	
	1				5					10					15		
10	Leu	Trp	Ala	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	
			20						25					30			
	Leu	Asp	Asn	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	
			35					40					45				
	Gln	Glu	Arg	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	
		50					55						60				
15	Pro	His	Arg	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	
	65					70					75					80	
	Met	Phe	Met	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	
				85						90					95		
20	Gly	Pro	Gly	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	
			100						105					110			
	Thr	Gln	Gly	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	
			115					120					125				
	Asp	Ala	Asp	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	
		130					135					140					
25	Glu	Phe	Phe	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	
	145					150					155					160	
	Ser	Lys	Ile	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	
				165						170					175		
30	Tyr	Lys	Asp	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	
			180						185					190			
	Ser	Val	Tyr	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	
			195				200						205				
	Phe	Leu	Leu	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	
		210					215					220					
	Val	Phe	Asp	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	
	225					230					235					240	
	His	Asn	Leu	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	
				245						250					255		
	Ile	Asn	Pro	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	

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05                    260                    265                    270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe  
                   275                    280                    285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser  
                   290                    295                    300

10 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu  
      305                    310                    315                    320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr  
                   325                    330                    335

15 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu  
      340                    345                    350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn  
                   355                    360                    365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His  
                   370                    375                    380

20 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln  
      385                    390                    395                    400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile  
                   405                    410                    415

25 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
      420                    425                    430

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1929 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 103..1293
- (D) OTHER INFORMATION: /function= "osteogenic protein"  
                               /product= "mOP2-PP"  
                               /note= "mOP2 cDNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGCT GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC

60



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05	CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT	114
	Met Ala Met Arg	
	1	
10	CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC	162
	Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly	
	5 10 15 20	
	GGC CAC GGT CCC GGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA	210
	Gly His Gly Pro Gly Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly	
	25 30 35	
15	GCG CGC GAC CGG GAC ATG CAG CGT GAA ATC CTG CCG GTG CTC GGG CTA	258
	Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro Val Leu Gly Leu	
	40 45 50	
	CCG GGA CGC CCC GAC CCC GTG CAC AAC CCG CCG CTG CCC GGC ACG CAG	306
	Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu Pro Gly Thr Gln	
	55 60 65	
20	CGT GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC	354
	Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp	
	70 75 80	
25	GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC	402
	Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val	
	85 90 95 100	
	ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG	450
	Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln	
	105 110 115	
30	GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT	498
	Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala	
	120 125 130	
	GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC	546
	Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser	
	135 140 145	
	ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC	594
	Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val	
	150 155 160	
	CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG	642
	Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln	
	165 170 175 180	
	ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA	690
	Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala	
	185 190 195	
	GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC	738

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05	Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg	200	205	210	
	CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT				786
	Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala	215	220	225	
10	GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA				834
	Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val	230	235	240	
	ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG				882
15	Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala	245	250	255	260
	AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC				930
	Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His	265	270	275	
20	CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC				978
	Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly	280	285	290	
	AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGA TTC CGT GAC CTT				1026
	Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Arg Phe Arg Asp Leu	295	300	305	
25	GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC				1074
	Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr	310	315	320	
	TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC				1122
30	Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr	325	330	335	340
	AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT				1170
	Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val	345	350	355	
35	GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG				1218
	Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val	360	365	370	
	CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC				1266
	Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn	375	380	385	
	ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC				1313
	Met Val Val Lys Ala Cys Gly Cys His	390	395		
	TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CTTTCTATGT				1373

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05 TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTCCTGCTA 1433  
 AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC 1493  
 CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA 1553  
 ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC 1613  
 CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGCC CTGGAATTCT AAACCTAGATG 1673  
 10 ATCTGGGCTC TCTGCACCAT TCATTGTGGC AGTTGGGACA TTTTATAGGTA TAACAGACAC 1733  
 ATACACTTAG ATCAATGCAT CGCTGTACTC CTTGAAATCA GAGCTAGCTT GTTAGAAAAA 1793  
 GAATCAGAGC CAGGTATAGC GGTGCATGTC ATTAATCCCA GCGCTAAAGA GACAGAGACA 1853  
 GGAGAATCTC TGTGAGTTCA AGGCCACATA GAAAGAGCCT GTCTCGGGAG CAGGAAAAAA 1913  
 AAAAAAACG GAATTC 1929

15 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 397 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(ix) FEATURE:  
 (D) OTHER INFORMATION: /Product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
 25 1 5 10 15  
 Ala Leu Gly Gly Gly His Gly Pro Gly Pro Pro His Thr Cys Pro Gln  
 20 25 30  
 Arg Arg Leu Gly Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro  
 35 40 45  
 30 Val Leu Gly Leu Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu  
 50 55 60  
 Pro Gly Thr Gln Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala  
 65 70 75 80  
 Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg  
 85 90 95



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05 Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
385 390 395

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 10 (A) LENGTH: 1941 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 15 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: HOMO SAPIENS  
 (F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:  
 20 (A) NAME/KEY: CDS  
 (B) LOCATION: 507..1703  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "hOP2-PP"  
 /note= "hOP2 (CDNA)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

25 GGAATTCCGG CCACAGTGGC GCCGGCAGAG CAGGAGTGGC TGGAGGAGCT GTGGTTGGAG 60  
 CAGGAGGTGG CACGGCAGGG CTGGAGGGCT CCCTATGAGT GGCGGAGACG GCCCAGGAGG 120  
 CGCTGGAGCA ACAGCTCCCA CACCGCACCA AGCGGTGGCT GCAGGAGCTC GCCCATCGCC 180  
 CCTGCGCTGC TCGGACCGCG GCCACAGCCG GACTGGCGGG TACGGCGGCG ACAGAGGCAT 240  
 TGGCCGAGAG TCCCAGTCCG CAGAGTAGCC CCGGCCTCGA GGCGGTGGCG TCCCGGTCCT 300  
 30 CTCCGTCCAG GAGCCAGGAC AGGTGTCGCG CGGCGGGGCT CCAGGGACCG CGCCTGAGGC 360  
 CGGCTGCCCCG CCCGTCCCGC CCCGCCCGCG CGCCCGCCGC CCGCCGAGCC CAGCCTCCTT 420  
 GCCGTGCGGG CGTCCCCAGG CCCTGGGTGCG GCCGCGGAGC CGATGCGCGC CCGCTGAGCG 480  
 CCCCAGCTGA GCGCCCCCGG CCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG 533  
 Met Thr Ala Leu Pro Gly Pro Leu Trp  
 35 1 5

CTC CTG GGC CTG GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG 581  
 Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu  
 10 15 20 25

CGA CCC CCG CCC GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAC CGG 629

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05	Arg Pro Pro Pro Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Asp Arg	30 35 40	
	GAC GTG CAG CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC		677
	Asp Val Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro	45 50 55	
10	CGG CCC CGC GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG		725
	Arg Pro Arg Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro	60 65 70	
	CTC TTC ATG CTG GAC CTG TAC CAC CGC ATG GCC GGC GAC GAC GAC GAG		773
15	Leu Phe Met Leu Asp Leu Tyr His Arg Met Ala Gly Asp Asp Asp Glu	75 80 85	
	GAC GGC GCC GCG GAG GCC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC		821
	Asp Gly Ala Ala Glu Leu Gly Arg Ala Asp Leu Val Met Ser Phe	90 95 100 105	
20	GTT AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT		869
	Val Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His	110 115 120	
	TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG		917
	Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala	125 130 135	
25	GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG		965
	Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu	140 145 150	
	CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG		1013
30	Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln	155 160 165	
	TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA		1061
	Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg	170 175 180 185	
35	GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC		1109
	Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp	190 195 200	
	TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG		1157
	Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val	205 210 215	
40	GAG ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG		1205
	Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu	220 225 230	
	GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC		1253
	Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe		

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05	235	240	245	
	AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG			1301
	Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu			
	250	255	260	265
10	AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA			1349
	Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg			
	270	275	280	
	CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC			1397
	Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Gln Val			
	285	290	295	
15	TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG			1445
	Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu			
	300	305	310	
	GAC TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG			1493
	Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly			
20	315	320	325	
	GAG TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC			1541
	Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala			
	330	335	340	345
25	ATC CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG			1589
	Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys			
	350	355	360	
	GCG TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT			1637
	Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr			
	365	370	375	
30	GAC AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC			1685
	Asp Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val			
	380	385	390	
35	AAG GCC TGC GGC TGC CAC TGAGTCAGCC CGCCCAGCCC TACTGCAGCA			1733
	Lys Ala Cys Gly Cys His			
	395			
	ATTCAGTGGC CGTCGTTTTA CAACGTGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA			1793
	TCGCCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCTAATA GCGAAGAGGC CCCGCACCGA			1853
	TCGCCCTTCC CAACAGTTGC GCCCCAGTGA ATGGCGAATG GCAAATTGTA AGCGTTAATA			1913
	TTTTGTAAAA ATTGCGGTTA AATTTTTT			1941

(2) INFORMATION FOR SEQ ID NO:6:





- 55 -

05 Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser  
 225 230 235 240

Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile  
 245 250 255

10 Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys  
 260 265 270

Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp  
 275 280 285

Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr  
 290 295 300

15 Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln  
 305 310 315 320

Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp  
 325 330 335

20 Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His  
 340 345 350

Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys  
 355 360 365

Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile  
 370 375 380

25 Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:7:

- 30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 35 (ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= OPX  
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS  
 AS DEFINED IN THE SPECIFICATION
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:



- 57 -

05 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys  
85 90 95

Xaa

10 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= PROTEIN

20 /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES  
ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER A-AMINO  
ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

25 Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa  
20 25 30

Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa  
35 40 45

30 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
50 55 60

Xaa Cys Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
85 90 95

Xaa Xaa Cys Xaa Cys Xaa  
100

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05      What is claimed is:

1. A polypeptide chain comprising an amino acid sequence described by residues 303-399 of Seq. ID No. 5.
2. The polypeptide chain of claim 1 comprising an amino acid sequence described by residues 297-399 of Seq. ID No. 5.
3. The polypeptide chain of claim 2 comprising of amino acid sequence described by residues 267-399 of Seq. ID No. 5.
4. The polypeptide chain of claim 3 comprising an amino acid sequence described by residues 264-399 of Seq. ID No. 5.
5. The polypeptide chain of claim 4 comprising an amino acid sequence described by residues 240-399 of Seq. ID No. 5.
6. The polypeptide chain of claim 5 comprising an amino acid sequence described by residues 1-399 of Seq. ID No. 5.
7. A polypeptide chain comprising an amino acid sequence described by residues of 301-397 of Seq. ID No. 3.
8. The polypeptide chain of claim 7 comprising an amino acid sequence described by residues 296-397 of Seq. ID No.3.

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- 05     9. The polypeptide chain of claim 8 comprising an amino acid sequence described by residues 259-397 of Seq. ID No. 3.
- 10     10. The polypeptide chain of claim 9 comprising an amino acid described by residues 1-397 of Seq. ID No. 3.
- 15     11. A polypeptide chain useful as a subunit of a dimeric osteogenic protein comprising a pair of disulfide-bonded polypeptide chains, said polypeptide chain having an amino acid sequence described by residues 303-399 of Seq. ID No. 5, including allelic and species variants thereof, such that the dimeric osteogenic protein comprising said polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a matrix.
- 20     12. The polypeptide chain of claim 11 wherein said amino acid sequence comprises residues 261-399 of Seq. ID 5.
- 25     13. The polypeptide chain of claim 11 wherein the amino acid sequence comprises residues 301-397 of Seq. ID No. 3.
- 30     14. The polypeptide chain of claim 13 wherein said amino acid sequent comprises residues 259-397 of Seq. ID No. 3.
15. A dimeric osteogenic protein capable of inducing endochondral bone formation in a mammal when implanted in said mammal in association with a matrix;

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- 05           said protein comprising a pair of disulfide-bonded  
polypeptide chains constituting a dimeric species,  
wherein each said polypeptide chain is the polypeptide  
chain of claim 11.
- 10           16. The polypeptide chain of claim 3 or 11 produced by  
expression of recombinant DNA in a host cell.
17. The polypeptide chain of claim 16 wherein said  
host cell is a procaryotic host cell.
18. The polypeptide chain of claim 16 wherein said  
host cell is a mammalian cell.
- 15           19. The polypeptide of claim 1, 3 or 11 that is  
glycosylated.
20. A nucleic acid encoding the polypeptide chain of  
claim 1, 3, or 11.
- 20           21. A dimeric protein comprising a pair of polypeptide  
chains expressed from a DNA sequence described by ID  
No. 3 or ID No. 5, including allelic and species  
variants thereof, such that, when said polypeptide  
chains are oxidized to produce a disulfide-bonded  
dimeric species, the dimeric species has a conformation  
25           that is capable of inducing endochondral bone or  
cartilage formation when disposed within a matrix and  
implanted in a mammal.

hOP2	Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg
mOP2	...	Ala	...	...	...	Lys	...	...
					5			
hOP2	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu
mOP2	...	...	...	...	Thr	...	...	...
		10					15	
hOP2	Pro	Gln	Ala	Asn	Arg	Leu	Pro	Gly
mOP2	...	His	Pro	...	Lys	...	...	...
				20				
hOP2	Ile	Phe	Asp	Asp	Val	His	Gly	Ser
mOP2	...	...	...	...	Gly	...	...	...
	25					30		
hOP2	His	Gly	Arg	Gln	Val	Cys	Arg	Arg
mOP2	Arg	...	...	Glu	...	...	...	...
			35					40
hOP2	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln
mOP2	...	...	...	...	...	Arg	...	Arg
					45			
hOP2	Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val
mOP2	...	...	...	...	...	...	...	...
		50					55	
hOP2	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala
mOP2	...	...	...	...	...	...	...	...
				60				
hOP2	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
mOP2	...	...	...	...	...	...	...	Ala
	65					70		

Fig. 1.1

hOP2	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn
mOP2	...	...	...	...	...	...	...	...
			75					80
hOP2	Ala	Thr	Asn	His	Ala	Ile	Leu	Gln
mOP2	...	...	...	...	...	...	...	...
				85				
hOP2	Ser	Leu	Val	His	Leu	Met	Lys	Pro
mOP2	...	...	...	...	...	...	...	...
		90					95	
hOP2	Asn	Ala	Val	Pro	Lys	Ala	Cys	Cys
mOP2	Asp	Val	...	...	...	...	...	...
			100					
hOP2	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr
mOP2	...	...	...	...	...	...	...	...
	105					110		
hOP2	Ser	Val	Leu	Tyr	Tyr	Asp	Ser	Ser
mOP2	...	...	...	...	...	...	...	...
			115					120
hOP2	Asn	Asn	Val	Ile	Leu	Arg	Lys	Ala
mOP2	...	...	...	...	...	...	...	His
				125				
hOP2	Arg	Asn	Met	Val	Val	Lys	Ala	Cys
mOP2	...	...	...	...	...	...	...	...
		130					135	
hOP2	Gly	Cys	His					
mOP2	...	...	...					

Fig. 1.2

SUBSTITUTE SHEET



hOP1	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
mOP1	...	...	...	Gly	...	...	...	...	...
hOP2	Ala	Val	Arg	Pro	Leu	Arg	...	Arg	...
mOP2	Ala	Ala	Arg	Pro	Leu	Lys	...	Arg	...
	1				5				

hOP1	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln
mOP2	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
	10					15			

hOP1	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
mOP1	...	...	...	...	...	...	Ser	...	...
hOP2	Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
mOP2	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
		20					25		

hOP1	Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Asp	Val	His	Gly	...	His	Gly	...	...
mOP2	Asp	Gly	His	Gly	...	Arg	Gly	...	Glu
			30					35	

hOP1	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Val	...	Arg	Arg	...	...	...	...	...
mOP2	Val	...	Arg	Arg	...	...	...	...	...
				40					45

Fig. 2.1

hOP1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	Gln	...	...	...	...	Leu	...
mOP2	Arg	...	...	...	...	...	...	Leu	...
					10				
hOP1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	Val	...	...	...	Gln	...	...	Ser
mOP2	...	Val	...	...	...	Gln	...	...	Ser
	55					60			
hOP1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	...	...	...	...	...	Ser
mOP2	...	...	...	...	...	...	...	...	...
		65					70		
hOP1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	Asp	...	Cys	...	...	...
mOP2	...	...	...	Asp	...	Cys	...	...	...
			75					80	
hOP1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	...	...	Leu	...	Ser	...
mOP2	...	...	...	...	...	Leu	...	Ser	...
				85					90

Fig. 2.2

hOP1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP1	...	...	...	...	...	...	Asp	...	...
hOP2	...	...	Leu	Met	Lys	...	Asn	Ala	...
mOP2	...	...	Leu	Met	Lys	...	Asp	Val	...

95

hOP1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	Ala	...	...	...	...	...	Lys
mOP2	...	...	Ala	...	...	...	...	...	Lys

100

105

hOP1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP2	...	Ser	...	Thr	...	...	...	...	Tyr

110

115

hOP1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP1	...	...	...	...	...	...	Asp	...	...
hOP2	...	Ser	...	Asn	...	...	...	...	Arg
mOP2	...	Ser	...	Asn	...	...	...	...	Arg

120

125

hOP1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP1	...	...	...	...	...	...	...	...
hOP2	...	Ala	...	...	...	...	...	Lys
mOP2	...	His	...	...	...	...	...	Lys

130

hOP1	Ala	Cys	Gly	Cys	His
mOP1	...	...	...	...	...
hOP2	...	...	...	...	...
mOP2	...	...	...	...	...

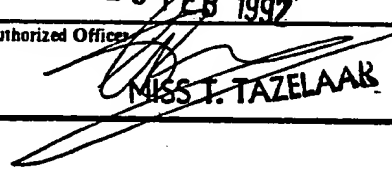
135

Fig. 2.3

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 91/07635

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.C1.5                      C 12 N 15/00                      C 07 K 7/10                      C 07 K 13/00 A 61 K 37/02                      A 61 K 27/00		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.C1.5	C 07 K                      A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X A	WO,A,8909788 (CREATIVE BIOMOLECULES) 19 October 1989, see the whole document ---	11,15- 21 1-10,12 -14
X A	WO,A,9011366 (GENETICS INSTITUTE) 4 October 1990, see the whole document ---	11,15- 21 11,15- 21
X	EMBO Journal, volume 9, no. 7, 1990, Oxford University Press (Eynsham, Oxford, GB) E. Ozkaynak et al.: "OP-1 cDNA encodes an osteogenic protein in the TGF-beta family", pages 2085-2093, see the whole article -----	11,15- 21
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<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
31-01-1992	20 FEB 1992	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 MISS J. TAZELAAR	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
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US 9107635

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		US-A- 5011691	30-04-91
		AU-A- 3444989	03-11-89
		AU-A- 3530589	03-11-89
		EP-A- 0372031	13-06-90
		EP-A- 0362367	11-04-90
		JP-T- 3500655	14-02-91
		JP-T- 3502579	13-06-91
		WO-A- 8909787	19-10-89
		AU-A- 5174790	26-09-90
		EP-A- 0411105	06-02-91
		JP-T- 3504736	17-10-91
		WO-A- 9010018	07-09-90
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		CA-A- 2030518	29-09-90
		EP-A- 0429570	05-06-91

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